UVL and the Epidermal Langerhans Cell

To the Editor:

In their interesting article published in the August 1985 issue, Hanau et al [1] reported that after UVB irradiation there is disappearance of the ATPase system of the epidermal Langerhans cell (ELC) and possible alteration of the absorptive pinocytosis system which in turn leads to interruption or alteration of the formation of ELC granules.

The authors state that there is controversy in the literature as to the nature of the effect of UVB on ELC. Some investigators, they say, claim that UVB depletes the ELC population [2,3] or that it depletes only their surface markers [4], while others believe that it damages the ELC cell membrane [5].

In one of our first works [5] we found that, on light microscopy (LM), disappearance and morphologic changes of ELC were directly proportional to the irradiation dosages. Electron microscopy (EM) of the areas irradiated with the *highest dosages* (4–6 MED) showed, indeed, clear disruption and fragmentation of the ELC cell membrane. In this connection the abstract of our preliminary work [5] was quoted as if we believe that the *only* effect of UVB upon ELC is to damage their cell membrane. In a subsequent study [6] we have also shown that differences exist according to the dosages administered and also the UV spectra used, i.e., UVA and UVB vs. UVC.

To arrive at their conclusion, Hanau et al [1] exposed 2.5 × 2.5 cm squares of previously shaved guinea pig flank skin to 44 mJ/cm^2 of UVB (approximately $\frac{1}{2}$ minimal erythema dose, MED) using a Westinghouse Sunlamp model FS 20 ($\pm$ λ313) on 4 successive days and obtained biopsies immediately following the last UVB exposure.

In our most recent work [7], we have performed multiple LM and EM examinations on guinea pig specimens that had received low (1–3 MED) and high (4–6 MED) dosages of UVB, λ310 through a Jasco CRM Fa spectroirradiator and have found that only high dosages of UVB are able to physically damage the ELC cell membrane, whereas lower dosages produce only disturbances of the spatial distribution of the ATPase loci of the ELC cell membrane, followed by their aggregation.

We wish to be quoted correctly in this regard, because we have clearly demonstrated (as explained above), surface marker depletion after low-dose UVB irradiation and cell membrane damage only after high-dose exposure [7]. We would like to add that we also have noticed a decreased number of ELC granules in irradiated ELC when compared with nonirradiated ones (unpublished data). This is in agreement with the findings reported by Hanau et al [1], who have seen a deficiency of ELC granules in UVB-irradiated, ATPase-negative ELC.

These findings give strength to the proposed theory that Birbeck granules derive from the ELC cell membrane [8–11]. Since UVB damages the activities of the ELC cell membrane and, at higher dosages, the ELC cell membrane itself, the formation of ELC granules at the cell membrane level is severely disturbed and this may account for the decreased number of ELC granules in UVB-irradiated ELC.

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REPLY

We would like to thank Dr. Iacobelli and Dr. Hashimoto for the precise details they give in their letter concerning the effects of UVB on epidermal Langerhans cells (LCs). At the time we submitted our article in May 1984 their work [1], published in 1985, was completely unknown to us. Moreover, we are most particularly pleased to note that they confirm all of our results, i.e., the disappearance of the LC enzymatic surface marker ATPase and the decrease in the number of Birbeck granules (we have often observed their disappearance and sometimes their deterioration) after low-dose UVB irradiation. We have also noted that Dr. Iacobelli and Dr. Hashimoto share our opinion concerning the fact that the decrease in the number of Birbeck granules or their disappearance might be the result of functional damage to the cell membrane. Some of the authors [2–4] quoted by Dr. Iacobelli and Dr. Hashimoto believe that Birbeck granules derive from the LC cell membrane, while Dr. Ishii et al [5] and Dr. Takigawa et al [6] hold that Birbeck granules would derive from the plasma membrane at the same time as coated pits and would be simply prolongations of the latter. However, we think that, under normal conditions, Birbeck granules appear in the cytoplasm, most probably arising from endosomes (receptosomes), these endosomes themselves being the result of an adsorptive pinocytosis [7]. According to that hypothesis rod-like (racket-like) Birbeck granules would be nothing more than the equivalent of the tubular (vesiculotubular) part of the CURL (compartment of uncoupling of receptor and ligand) [8]. This difference of opinion as to the origin of Birbeck granules should not diminish the

importance of the fact that our 2 groups observe, after low-dose UVB, similar LC lesions.

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Effectiveness of Azelaic Acid As a Depigmenting and Chemotherapeutic Agent

To the Editor:

At first sight it might appear that the findings reported by Pathak et al in the September issue of the Journal (pp 222–228) prove that azelaic acid has no value as a chemotherapeutic agent, and that they contradict observations of ours, and others, published over the past 7 years, and extended by our report published in the same issue (pp 216–221). In fact, the results of the Boston group's study entirely confirm what we have been emphasizing all along, i.e., that azelaic acid has little or no effect on the majority of normal melanocytes in vivo (a 20% topical cream) or in vitro at concentrations of 10^{-3} M, or less. We have also reported (J Invest Dermatol 82:542, 1984) that at 10^{-3} M it has no effect on melanoma cells in culture, and it is evident from the graphs in Fig 1 of our current paper that at this concentration it has, in fact, a stimulating effect on growth of Harding-Passey and Cloudman cells in culture. It is only at higher concentrations ($>10^{-3}$ M) that we find an effect on cell proliferation and/or viability, and the Boston group and Leibl et al (Arch Dermatol Res 276:273a, 1984) also found this. All 3 groups supplement their culture media with fetal calf serum, and in this connection it may be pointed out that Schachtschabel (Proceedings of the Vth European Workshop on Melanin Pigmentation, 1984, p 43) reported a significant inhibition of growth of Harding-Passey melanoma cells with azelaic acid at 1×10^{-3} M. It might be of interest for the other 3 groups mentioned to repeat their experiments using serum-free medium.

Pathak et al suggest, but do not substantiate with data, that the effect on growth and viability observed by them with azelaic acid at 10^{-2} M results from "perturbations of hydrogen ion concentration." In our present paper we conclusively show that the effects are not due to "perturbed" pH, or high osmolarity, and that another dicarboxylic acid, adipic acid (C_6) at similar concentrations, pH, and osmolarity, in similar medium, does not produce the same effects as azelaic acid; Leibl et al reported the same lack of effect with adipic acid, confirming the selective effect of azelaic acid. They also confirmed its beneficial clinical effect on lentigo maligna.

Pathak et al state that they have found all putative melanocytotoxic agents to be invariably toxic for both normal and abnormal melanocytes, and express surprise that azelaic acid should be selective in this respect. It is—and this is one of its main advantages over other agents, leading to the cosmetically acceptable result in, for example lentigo maligna and cholasma—that there is no residual hypochromia at the site of the resolved lesion. Many of the agents referred to by them such as 4-OHA are substrates of tyrosinase and are used up, leading to production of toxic intermediates which damage melanocytes. Azelaic acid is a competitive inhibitor of tyrosinase, and is not used up. It acts, in fact, as we have reported (Passi et al, Biochem Pharmacol 33:103–108,1984) against the mitochondrial oxido-reductase enzymes, and our suggestion that it might inhibit DNA synthesis has been confirmed by Leibl et al and more recently by ourselves (Reith et al, IRCS Med Sci 13:783–784, 1985). We are gratified to receive further confirmation on this point by "laboratory studies in progress" in Boston. This question of a selective effect on abnormal melanocytes is, of course, important to understand. A recent study of ours (Picardo et al, Biochem Pharmacol 34:1653–1658, 1985) on normal lymphocytes and lymphoma- and leukaemia-derived cell lines in culture, suggests it may be related to differences in permeability of the cell membrane.

Many of the other points raised by Pathak et al have already been dealt with in publications of ours listed in their bibliography, to which we refer your readers. Papers by Robins et al (Br J Dermatol 113:687–697, 1985), Leibl et al (J Invest Dermatol 85:417–422), Hu et al (Br J Dermatol 114:17–26, 1986), and Geier et al (Hautarzt 37:146) are highly relevant to and supportive of the points made above.

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